CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF HUMIC SUBSTANCES

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1. Problems concerning the characterization of the chemical composition and of the physical properties of humic substances.

The characterization of the chemical composition and physical properties of humic substances is difficult, because these substances are formed, by and large, by reactions as yet unknown and hitherto no substances of a uniform chemical structure have been isolated. The literature shows that substances of the same type have seldom been analyzed. Elucidation of the properties of humic substances is complex for the following reasons:

1. The composition of the isolated substances is variable because they are formed from different initial materials.
2. Often different methods are used for the isolation of fractions of soil organic matter, so for instance the "humus acids". Only one attempt has been made to identify a certain fraction as "humic acids", which should be isolated by a conventionally fixed method /Flaig 1964/.

Differences are found in the use of methods for the characterization of chemical and physical properties. These differences are variable according to the origin of the "humic acids". It would be desirable to identify the monomers, which participats in the formation of humic acids, and the linkages, which exist between the various monomers. The difficulties mentioned under 1. and 2. would decrease if selective reactions were to occur during the formation of humic substances. There may be some indications for the occurence of selective reactions

At the beginning it should be mentioned that the results described concerning the chemical and physical properties of humic substances are obtained from preparations which have been isolated from different soils in different ways. However, only the values obtained with conventional methods are suitable for comparison and can be used for reviews.

The purpose of my lecture is not only to enumerate the chemical and physical properties of humic substances but also to explain the reactions which lead to these. Time allows us to mention only some problems which either seem to me actual or which are not reported in the following communications. Recently, important specialised literature such as books /Kononowa 1966, McLaren 1967, report of IAEA-FAO 1966/ or articles in handbooks /Flaig 1966/ have been published about the state of research in the fields of humic substances.

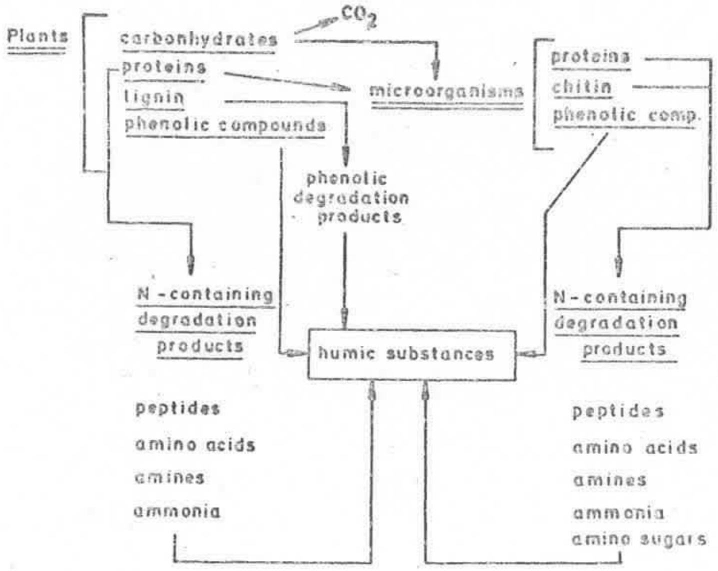


Fig. 1.: Scheme of transformation of constituents of plants and microorganisms during humification.

The existing knowledge about the composition of humic acids supports the view that reactions between phenols derived from lignin or synthesized by microorganisms and nitrogenous compounds derived from proteins are important in the formation of humic substances. Other groups of substances have not yet been isolated from humic acids in a comparable amount. The further considerations are in connection with this subject.

The theory that humic acids are formed by condensation of phenolic and nitrogenous compounds has developed from two types of work. Fragments from the degradation of humic acids have been isolated as chemical compounds, and experiments imitating the synthesis of humic acids under artificially imposed physiological conditions have been performed in test tubes or in cultures of microorganisms. Essential questions are not yet answered; recently, however, new results concerning the chemical composition of humic acids have been obtained through the use of 14 carbon labelled compounds. What is more, some steps of the mechanism of formation of humic acids could be elucidated using this technique. But, here also we are only at the beginning.

2. Phenols as materials for the composition of humic acids.

2.1 Identification of phenols as structural units of humic acids.

Regarding the participation of phenols in the structure of humic acids it will be remembered that phenolic compounds are obtained by oxidative cleavage in alkaline nitrobenzene solution /Morrison 1963, Farmer and Morrison 1964/ or by degradation with alkaline potassium permanganate solution /Schnitzer and Desjardins 1964, Wright and Schnitzer 1959, Kumada, Suzuki and Aizawa 1961/, as well as by reductive cleavage of humic acids with sodium amalgam /Burges, Hurst and

Walkden 1964, Burges, Hurst, Walkden, Dean and Hirst 1963/. Yields of 30 % of the carbon of the hydrolized portion of humic acids are reported after the reductive cleavage /Burges et al. 1964/. Other authors could not verify this result in their investigations /Mendez and Stevenson 1966/. Using model substances we established that different polyphenols are changed into several other phenols /Salfeld unpublished/. Also a small yield of phenolic degradation products after oxidative or reductive cleavage is not conclusive evidence that only few aromatic units are present in humic acids. The yield of vanillin after the oxidation of coniferous lignin with nitrobenzene in alkaline solution is only 25 %, although coniferous lignin is nearly a pure polymer of coniferyl alcohol. A pretreatment with acid or alkali as well as the presence of other plant constituents decreases the yield to a few percent.

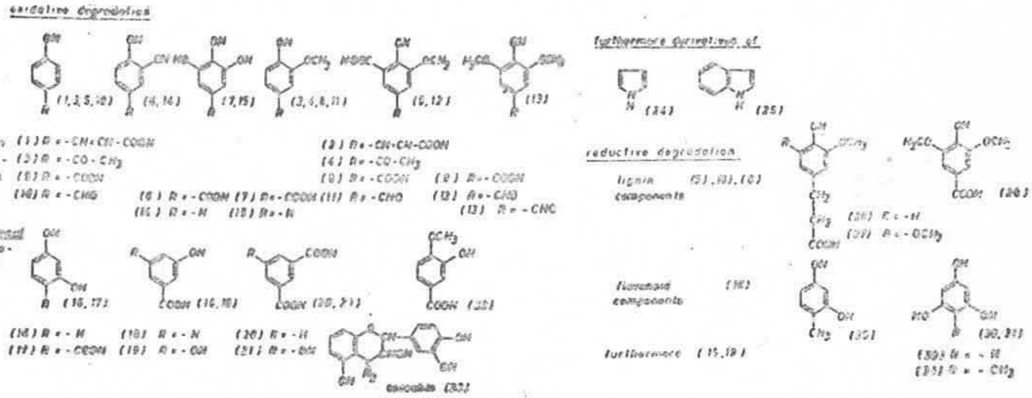


Fig. 2.: Degradation products of humic acids.

The phenols derived from lignin are mainly compounds of the guajacyl (2,4, 8,11,26) or syringyl type (7,9,12,13,15,27,28) or transformation products of p-coumaryl alcohol (1,3,5,10). Moreover, some other compounds are mentioned which are formed after the cleavage of the methyl ethers e.g. (6,14 and 7,15). The derivatives of resorcinol (16,17,29) or of phloroglucinol (30,31) may be derived from flavanoids according to Burges et al. /1964/ or are synthesized by microorganisms according to Haider and Martin /1967/.

2.2 Participation of lignin and its degradation products in composition of humic acids.

To understand the variations in chemical composition and physical properties of fractions isolated from humic substances, the corresponding transformations of lignin as one of the important initial materials for the formation of humic substances, are briefly discussed.

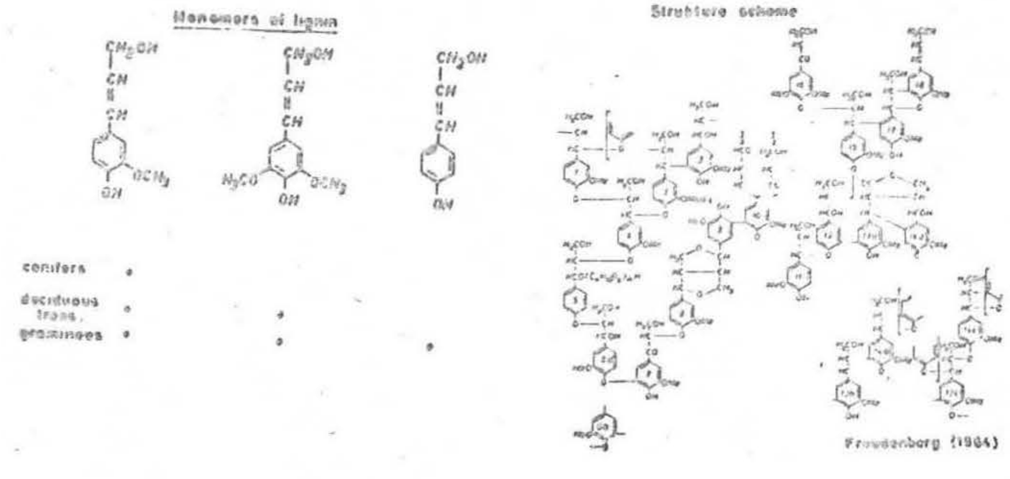


Fig. 3.: Lignin units and structure scheme of coniferous lignin according to Freudenberg /1964/ and Freudenberg and Harkin /1964/.

Freudenberg /1964/ and Freudenberg and Harkin /1964/ have published a structure scheme of coniferous lignin, in which the units of coniferyl alcohol are connected three-dimensionally to a high molecular weight compound. During the biological degradation of lignin, carbon-carbon or carbon-oxygen linkages are split off and other, mostly oxidative transformations occur.

According to some investigations low polymer degradation products of lignin participate in the formation of humic acids.

Otherwise, it is not certain how smaller monomer degradation products of lignin participate in the formation of humic acids. Using labelled lignin degradation products such as phenol carboxylic acids and phenol acrylic acids as well as their nitrogen free polymers formed by oxidative polymerisation in the presence of phenol oxidases, we found that these compounds are relatively easily degraded by lignin decomposing soil fungi and that they can be utilized as a carbon source by the microorganisms /Flaig and Haider 1961, Haider, Lim and Flaig 1962, Haider 1965/.

In contrast to this, the nitrogenous polymers of lignin degradation products are more resistant to the microbial degradation. Therefore, the possibility exists that these substances accumulate in the soil. The nitrogen containing condensation products have properties that are comparable with those of humic acids isolated from soil.

Tab.1. Elementary composition of lignin, humified lignin, humic and fulvic acids.
(accord.to Freudenberg and Harkin 1964, Maeder 1960, Kononowa 1966[1)], Dragunov 1948[2)])

Lignins		% C	% H	% O	% OCH_3	% N
1) Beech (Fagus silvaticus)	$C_9H_{6{,}5}O_2(H_2O)_{0{,}64}(OCH_3)_{1{,}41}$	61,87	6,03	32,10	21,65	
2) Spruce (Picea exelsa)	$C_9H_{7{,}12}O_2(H_2O)_{0{,}40}(OCH_3)_{0{,}92}$	65,08	5,88	29,04	12,87	
3) Peat-moss (Sphagnum)	$C_9H_{7{,}96}O_2(H_2O)_{0{,}90}(OCH_3)_{0{,}25}$	61,67	5,89	32,44	4,36	
4) Rye straw (Secale cereale)	$C_9H_{6{,}51}O_2(H_2O)_{1{,}05}(OCH_3)_{1{,}13}N_{0{,}08}$	63,10	5,92	30,67	17,20	0,54
Humified lignin						
5) Rye straw decomposed 180 days	$C_9H_{5{,}45}O_2(H_2O)_{1{,}42}(OCH_3)_{0{,}68}N_{0{,}24}$	61,15	5,42	32,42	11,05	1,75
Humic acids						
Fulvic acids						
6) Northern podzol under forest[1)]		58,11 52,37	5,37 3,33	32,00 42,89	-	4,52 1,21
7) Podzolic soil[2)]		57,94	5,79	31,41	1,54	4,86
8) Ordinary chernozem, arable[1)]		62,13 44,84	2,91 3,45	31,38 49,36	-	3,58 2,35
9) Chernozem[2)]		57,32	4,25	34,39	1,17	4,04

The elementary composition of lignin and humic acids demonstrate that the carbon content of the lignins of different plants is higher than that of humic acids. The decrease in carbon content and increase in oxygen content show that oxidative processes play an important role during the transformation of lignin into humic acids. Furthermore, the nitrogen content increases, whilst the content of methoxyl groups decreases remarkably during humification.

We could show with specifically labelled synthetic lignins and with polymers of lignin degradation products that the cleavage of the methylether occurs relatively fast by the action of lignin decomposing fungi, because the rate of the released labelled carbon atoms of the methoxyl groups was comparatively high /Haider and Lim 1965/.

The different content of methoxyl groups of lignin and humic acids indicate that a remarkable cleavage of the methylether of the lignin must have occurred, if lignin is to be regarded as an initial material of humic acids, and also other phenols participate in the formation of humic acids which have no methoxyl groups as it follows also from the mentioned degradation experiments.

If the constituents of lignin participate to a larger extent in the formation of humic acids, the cleavage of the methylether (comp. fig. 6) may be regarded as a selective reaction during humification.

2.3 Participation of phenols of microbial origin in the composition of humic acids.

A further source of phenols is the metabolism of some microorganisms. In this case low molecular weight compounds are formed and react to yield high molecular weight substances.

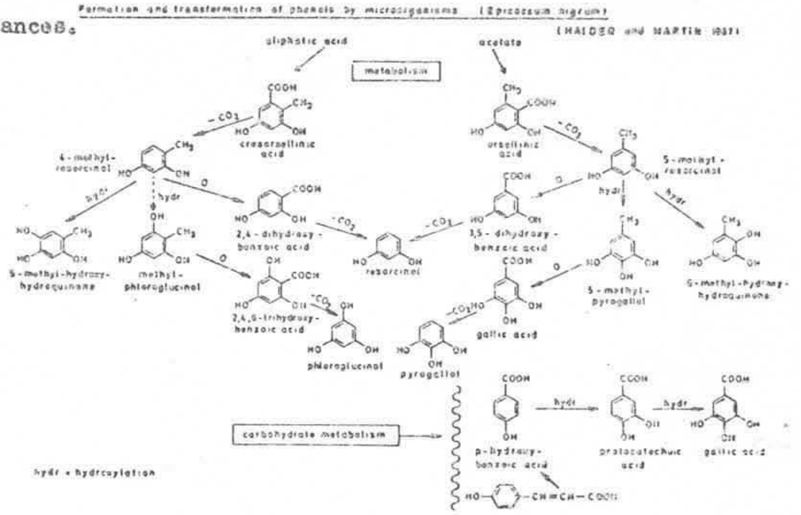


Fig. 4.: Formation of phenolic compounds by Epicoccum nigrum and their transformation.

The formation of different phenols in the culture of Epicoccum nigrum is reported as an example for research into the microbial synthesis of humic acids. These phenols are also partly identified in the humic acids formed by this fungus after reductive cleavage /Haider and Martin 1967, Martin, Richards and Haider 1967/. The fungus synthesizes orsellinic acid and cresorsellinic acid from aliphatic compounds. Different phenols are formed from both these acids by decarboxylation, by oxidation of the methyl group to a carboxyl group and by hydroxylation.

Furthermore the fungus also synthesizes small amounts of compounds which have the same constitution as some lignin degradation products.

It must be noted that 5- and 6-methylhydroxy-hydroquinone, gallic acid, pyrogallol and 5-methylpyrogallol are formed as autoxydisable compounds. The formation of these compounds is a prerequisite for the formation of humic acids in the culture of the fungus.

In this respect, it must also be mentioned that hydroxyhydroquinone is formed after the enzymatic oxidation of different lignin degradation products in microbial cultures to protocatechuic acid and by further oxidation of this acid in the presence of phenol oxidases /Flaig and Haider 1961, Haider, Lim and Flaig 1964/. These derivatives of polyphenols can be oxidized to quinones in the culture media at pH-values of 6 to 8, whilst this is not the case with derivatives of resorcinol and phloroglucinol.

2.4 Different reactivity of phenols participating in the composition of humic acids during the addition of nitrogen compounds.

Among the identified phenols which are formed either by oxidative or reductive cleavage of humic acids, or isolated from soils or synthesised by microorganisms, there are two types of phenolic compounds, which are of interest in studies of the structure and therefore also of the chemical and physical properties of humic acids.

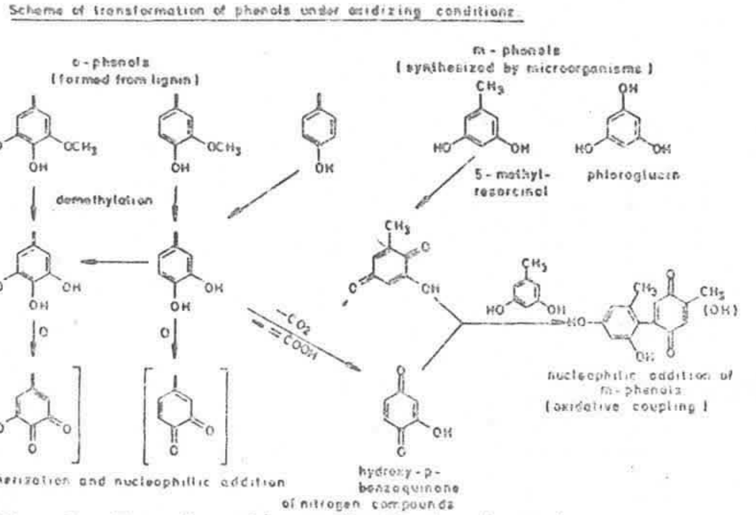


Fig. 5.: Transformations of polyphenols under oxidizing conditions.

Phenols have been found which possess

either two or several hydroxyl groups in o-position

or

two or three hydroxyl groups in m-position.

Both types of compounds differ widely in their reactivity. Catechol hydroxyhydroquinone and pyrogallol derivatives are distinguished from the derivatives of resorcinol and phloroglucinol derivatives by the fact that they dimerise and add nucleophilic proteins or their products of hydrolysis during the oxidation to quinones.

The differences in reactivity are important with regard to the function of nitrogen in the molecule of humic acids. Otherwise the formed quinones add nucleophilic resorcinol or phloroglucinol derivatives /Musso et al. 1965/. This oxidative coupling leads to ramification and increases aromaticity.

3. Nature of nitrogen in humic acids.

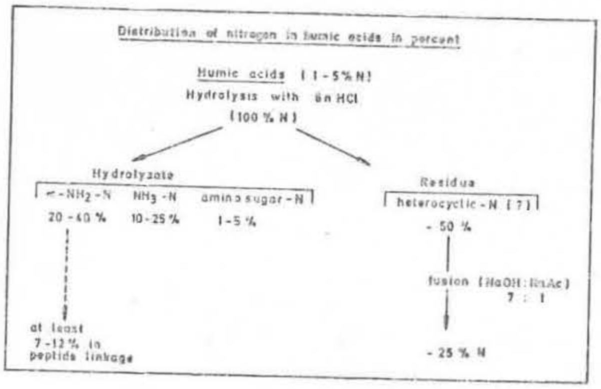


Fig. 6.: Function of nitrogen in humic acids.

It is known from numerous investigations (summary: Bremner 1955, Flaig 1966, p.403-404) that 30-50 % of the humic acid-nitrogen can be hydrolized with 6 N hydrochloric acid. From 20 to 40 % of the total nitrogen in the humic acid hydrolysate consists of α-amino-nitrogen, while 10 to 25 % is NH$_3$-nitrogen, referred to the total nitrogen of humic acids. According to the experiments of Bremner /1955/ and Young and Mortensen /1958/ 1 to 5 % of the total nitrogen is amino sugar nitrogen. The α -amino-nitrogen is derived mostly from microbial protein (Kuo and Bartholomew 1966).

About 50 % of the original nitrogen remains in the residue of the hydrolysis. Most authors postulate that this nitrogen is bound in heterocyclic form. Of this nitrogen, about one-half is split off as ammonia by fusion with a mixture of sodium hydroxide and sodium acetate (ration 7:1) in an iron crucible at 270^0 for 7 minutes. On the basis of model experiments with N-phenylglycine, we assume that this nitrogen consists partly of non-hydrolizable amino acids /Flaig and Breyhan 1956/. Th small variations in the quantitative composition of hydrolizable amino acids (comp. Scharpenseel and Krausse 1962) may be considered as a selective reaction in the sense mentioned before. The fact that tryptophane was not released from humic acids by different degrees of hydrolysis may also be interpreted in the same manner.

Scharpenseel and Krausse /1962/ found by treatment of ^{14}-carbon labelled humic acids with papain, an enzyme which splits off peptide linkages, that at least 30 % of the hydrolizable amino acids are bound as peptides.

3.1 About the linkage of amino acids, peptides and proteins in humic acids.

3.11 Nucleophilic addition of nitrogen compounds by phenols during oxidation.

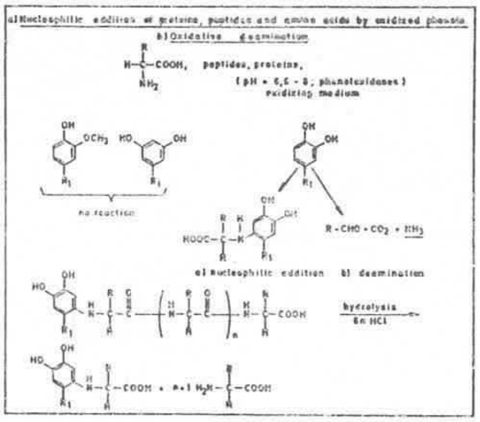


Fig. 7.: a) Nucleophilic addition of proteins, peptides and amino acids by phenols during oxidation.
b) Oxidative deamination.

We made extensive studies of the possible linkage of proteins and of their products of hydrolysis to phenols during oxidation with labelled amino acids /Haider, Frederick and Flaig 1965/. Some lignin degradation products isolated from lignin degradation reactions or from soils as well as some phenols of microbial origin can add nitrogenous compounds derived from proteins at variable rates and in different amounts during oxidation in the presence of phenol oxidases.

To sum up, we established that guajacol- and resorcinol derivatives do not add amino acids in a pH range of 6,5 to 8,0. On the other hand, varying amounts of amino acids are subjected to nucleophilic addition by catechol and hydroquinone derivatives. Addition by pyrogallol derivatives also occurs but to a smaller extent, depending on the chemical constitution of the derivatives. It could be proved by means of labelled compounds that the amino acids are added intact. This addition also occurs with peptides and proteins.

Furthermore, during oxidation in the presence of phenol oxidases and nitrogen compounds, catechol derivatives polymerize to nitrogenous polymers which have properties comparable to those of humic acids. In contrast, the polymers of guajacol derivatives do not contain nitrogen /Flaig and Haider 1961/.

The added amino acids cannot be hydrolyzed from the corresponding addition products by 6 N hydrochloric acid. However, in the case of addition products formed with proteins and peptides, all the amino acids could be hydrolyzed except the N-terminal amino acid in which the amino group has reacted with the oxidized phenol /Haider, Frederick and Flaig 1965/. Therefore not all non-hydrolizable nitrogen is bound in heterocyclic form as is often supposed.

3.12 Oxidative deamination of amino acids.

According to the chemical constitution of the phenols, not only an addition but also a deamination of the amino acids occurs. The amino acids are split off to give the corresponding aldehydes, ammonia and carbon dioxide. The ammonia produced in this reaction serves as a reactant in humification. We could show that the above mentioned catechol, hydroquinone or hydroxyhydroquinone derivatives do not add ammonia at pH values between 6 and 8 during the enzymatic oxidation of these phenols /Haider, Frederick and Flaig 1965/. A reaction with ammonia occurs under oxidizing conditions only when the pH values are higher than 8. Dark coloured, nitrogenous substances with properties comparable to those of humic acids are formed only under these conditions. Eller /1921/ was the first to synthesize humic acids from hydroquinone, hydroxyhydroquinone or pyrogallol in this way.

3.13 Possibilities of participation of heterocyclic nitrogen compounds in composition of humic acids.

Using amino acids labelled either in the carboxyl group or in another carbon atom, we have shown that ammonia and carbon dioxide are not split off in a molar ratio of 1:1 in all cases. A 1:1 ratio would be expected for the reaction of various o-diphenol with amino acids in the presence of phenol oxidases under oxidizing conditions according to the equation for the oxidative deamination reaction /Haider, Frederick and Flaig 1965/. More carbon dioxide is split off than ammonia is released. Some addition products can be precipitated by mineral acids and have a higher nitrogen content than that corresponding to the addition of one amino acid by one phenol.

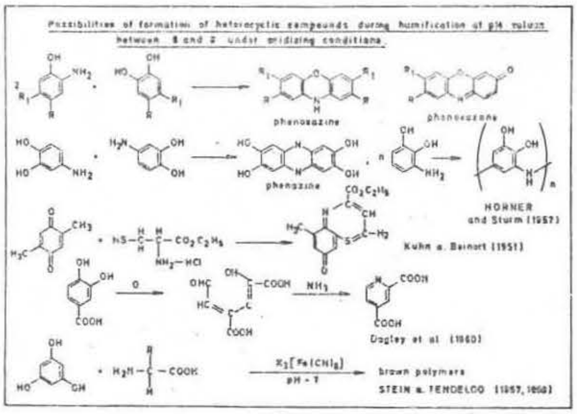

Possibilities of formation of heterocyclic compounds during humification at pH values between 6 and 8 under oxidizing conditions.
phenoxazine
phenoxazone
phenazine
HORNER and Sturm (1957)
Kuhn a. Beinert (1951)
Dagley et al (1960)
brown polymers
STEIN a. FENDELOO (1957, 1958)
pH - 7

Fig. 8.: Possibilities of formation of heterocyclic compounds during humification at pH-values between 6 and 8 under oxidising conditions.

We explained the reaction mechanism of addition and deamination of amino acids in the presence of catechol derivatives in an oxidising medium with reference to the work of Trautner and Roberts /1950/. In this case, the possibility exists that o-amino phenols are formed. Phenoxazines may be formed by condensation with the original catechol derivatives under oxidizing conditions. In the case of hydroxyhydroquinones, phenoxazones are formed. Further reactions of this type occuring under natural conditions are described for instance by Butenandt, Bickert and Schäfer /1960/. Horner and Sturm /1957/ have demonstrated that 4-amino catechol reacts with itself to a phenazine derivative, and 3-amino catechol condenses to polymers.

Heterocyclic condensation products are also formed by condensation reactions between quinones and amino acids which contain sulphur. Kuhn and Beinert /1944/ described the condensation of 2,5-dimethyl-p-benzoquinone with the ester of cystein to a dark coloured, heterocyclic compound, which contains nitrogen and sulphur in one ring.

Nitrogen ring compounds such as pyridine derivatives are formed after the oxidative cleavage of catechol or protocatechuic acid through the half-aldehyde of carboxymuconic acid /Trippett, Dagley and Stopher 1960, Dagley, Evans and Ribbons 1960/.

The formation of brown polymers by the reaction of phloroglucinol with amino acids in the presence of potassium ferricyanide is also described. Phloroglucinol cannot be oxidized to a quinone and therefore the usual nucleophilic addition of amino acids cannot occur. Stein and Tendeloo /1955, 1956a,b, 1958, 1959/ suppose that the first step of the reaction is the formation of a free radical.

Phenoxazones are also formed by the reaction of resorcinol derivatives such as orcinol with ammonia under oxidizing conditions but only at pH values above 8 /Musso and coworkers 1961, 1963, 1965/.

4. Nature of oxygen in the humic acids.

The oxygen atoms in the molecule of humic acids are distributed among different functional groups. The carboxyl groups are formed by various reactions, for instance, by the oxidative cleavage of the aromatic rings. Haider and Grabbe /1967/ have investigated different types of lignin synthesized by the method of Freudenberg /1956, 1962/. The lignin units were labelled in the carbon atoms of methoxyl groups or side chains or rings. The cleavage of the aromatic ring of lignin was found to be rapid during degradation by microorganisms and therefore to be an essential reaction in its microbial degradation. In addition to the cleavage of the ring, a degradation of the single carbon atoms of the side chains was measured. In both cases, the formation of carboxyl groups accurs. Other possibilities for the formation of carboxyl groups by oxidation of methyl groups have been mentioned in the review of participation of phenols of microbial origin in the formation of humic acids. Furthermore humic acids contain carboxyl groups of amino acids.

Tab.2.: Analytical characteristics of a humic and fulvic acid, according to Schnitzer /1965/.

Elementary composition		
Element	Humic acid (%)	Fulvic acid (%)
C	56,72	50,92
H	5,21	3,34
N	2,37	0,74
S	0,35	0,26
O (by difference)	35,35	44,74
Oxygen containing functional groups (m-equivalent per g dry ash-free material)		
Total acidity	5,7	12,4
Carboxyl	1,5	9,1
Total hydroxyl	6,9	6,9
Phenolic hydroxyl	4,2	3,3
Alcoholic hydroxyl	2,7	3,6
Carbonyl	0,9	3,1
Molecular weight x)	1684	669

x) The average molecular weights were determined by Schnitzer and Desjardins /1962/ by lowering of freezing point in sulfolane.

Another portion of the oxygen equivalents is fixed in phenolic or alcoholic hydroxyl groups. Finally, a larger amount of oxygen equivalents is bound as ether groups (e.g. Martin, Dubach, Mehta and Deuel 1963). A small amount of this can be easily determined as methylether of phenols; however, the form in which the ether linkages are present in humic acids, in contrast with that of their distribution in lignin is not yet known /Adler 1961, Freudenberg 1964, Freudenberg and Harkin 1964/. Attempts to cleave diphenylether linkages, which could have been present, with sodium in pyridin according to the method of Musso, Gizycki, Krämer and Döpp (1965/ were unsuccessful in the case of humic acids formed by fungi /Haider and Martin 1967/.

By various titration procedures (summary: Kononowa 1966, page 60-64, von Dijk 1967, Posner 1964/ differences between weak and strong acid groups can be detected. A quantitative differentiation in carboxyl or phenol groups is not possible. The titration procedure will not be mentioned in detail and the methods of characterizing the phenolic hydroxyl groups and carboxylic groups will not be discussed. Schnitzer and Desjardins /1965/ used the findings of former authors and tested the methods with model substances. Although there is much data on the distribution of the function of oxygen, which could be discussed; only the data of Schnitzer and Desjardins will be given as an example for the composition of humic and fulvic acids from podsol and for the distribution of functional groups (comp. fig. 2).

Similar investigations concerning the characterization of humic acids of different origin have been published recently by other authors /Fischer, Schlungbaum and Kadner 1964, Fischer, Schlungbaum and Belau 1964, Fischer, Schlungbaum, Belau and Preu 1965, Kasatochkin, Kononowa, Larina and Egorova 1964, Ziechmann 1964, Kröger, Darsow and Fuhr 1965, Kröger 1966, Schnitzer and Skinner 1966/. The review of the relatively voluminous literature demonstrates that the distribution of the oxygen function in humus differs not only according to origin but also according to the method of isolation. However, the results show principally similar tendencies.

Carbonyl groups have also been determined in humic acids. The methods for the determination of these functional groups with carbonyl reagents such as 2,4-dinitrophenylhydrazine, phenylhydrazine, semicarbazide and hydroxylamine involve the assumption that the determined carbonyl groups belong mainly to aliphatic parts of the molecule of humic acids and not to quinonoid groups. Not all quinones react with the above reagents since the reaction is dependent upon the substitution of the quinones.

For further considerations, it should be mentioned that humic acids contain carbonyl groups and different strong acidic groups, which are partly phenolic hydroxyl or carboxylic groups. Furthermore, it should be mentioned that nitrogen as well as sulphur is present in the molecule of humic and fulvic acids. The above characteristics may also have an influence on the colour of humic acids.

5. Colour and electronic spectra of humic and fulvic acids.

5.1 Possible chromophoric groups.

The previously mentioned reactions, which could result in the formation of heterocyclic structural units, lead also to considerations of the colour of humic acids. .

For this purpose, all units of the molecule which absorb only in the ultraviolet light can be excluded. The aliphatic portions of acids, proteins and amino acids are such units, provided they are not linked with quinones through an amino group. Phenols absorb only in the ultraviolet. Lignin is a typical example. Its absorption maximum between 275 and 285 nm is attributed to the benzene nucleus with oxygen containing groups, and the shoulder between 300 to 350 nm to the presence of chromophoric groups such as carbonyl groups or double bonds in the side chain (Jones 1949, Schobinger 1958).

5.11 Quinones.

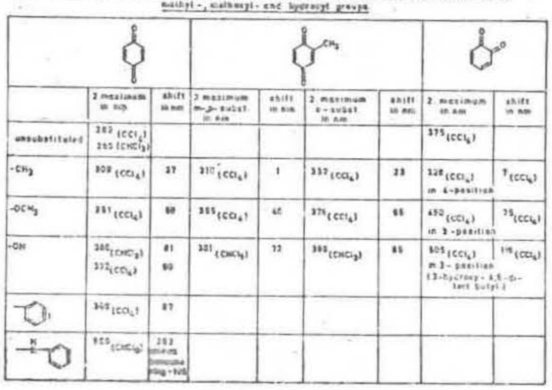
The absorption of humic acids in the visible range may be caused by chromophoric groups which are formed by oxidation of phenolic hydroxyl groups to carbonyl groups. IR-spectra lead us to assume that quinonoid groups are present in the molecule of humic acids. Exact determination of the wave length and the strength of absorption of conjugated C=O and C=C-groups is difficult in the case of quinones in mixtures with similar absorbing substances, because the absorption strength and range of the quinones vary with the substitution /Flaig and Salfeld 1959, Flaig 1960/.

Furthermore quinones or their corresponding phenols such as methoxy-p-benzoquinone /Sundman and Haro 1966/ or hydroxyhydroquinone /Flaig and Haider 1961/ have been identified during enzymatic oxidation of lignin degradation products in cultures of lignin decomposing fungi or in the presence of phenoloxidases.

Also we could demonstrate /Haider and Martin 1967/ that the formation of humic acids depends upon the transformation of microbial synthesized resorcinol derivatives to hydroxyhydroquinone or pyrogallol derivatives. Condensation to humic substances through semiquinone radicals as intermediates occurs only by further oxidation.

But generally speaking the quinones of oxidized phenols from lignin degradation or from microbial synthesis cannot be found in the same amount as the corresponding phenols, because this type of quinone has a relatively high redox potential and is reduced by oxidizing the substituted, the dimerized or the polymerized phenols, which have lower redox potentials of about 80 to 200 mV. The further condensation reactions proceed, the lower will be the redox potential. Only partial oxidation of the polymers occurs and the resulting substances become intramolecular quinhydrones. The redox polymers insist upon a quinhydrone state, as could be demonstrated in the case of polyvinyl-quinhydrone by redox titration /Cassidy 1949, Updegraff and Cassidy 1949/.

Aliphatic portions, methoxyl groups, phenolic hydroxyl groups and perhaps substituted benzene rings and primary or secondary amino groups may be important substituents of quinones, which are formed by the oxidation of phenolic units of humic acids. Carboxyl groups as substituents of quinones can be excluded, because o-quinone carboxylic acids are decarboxylated very easily and p-quinone carboxylic acids are very unstable.



Shift of the 2. maximum of absorption spectra of o- and p-benzoquinones by substitution with methyl-, methoxyl- and hydroxyl groups

	2. maximum in nm	shift in nm	2. maximum m-,p-subst. in nm	shift in nm	2. maximum o-subst. in nm	shift in nm	2. maximum in nm	shift in nm
unsubstituted	282 (CCl4) 285 (CHCl3)						375 (CCl4)	
-CH3	309 (CCl4)	27	310 (CCl4)	1	332 (CCl4)	23	382 (CCl4) in 4-position	7 (CCl4)
-OCH3	351 (CCl4)	69	355 (CCl4)	46	374 (CCl4)	65	450 (CCl4) in 3-position	75 (CCl4)
-OH	366 (CHCl3) 372 (CCl4)	81 90	381 (CHCl3)	72	394 (CHCl3)	85	505 (CCl4) in 3-position (3-hydroxy-4,6-di-tert-butyl)	115 (CCl4)
	369 (CCl4)	87						
	525 (CHCl3)	243 [illegible]						

Fig. 9.: Shift of the 2. maximum of absorption spectra of o- and p-benzoquinones by substitution with methyl-, methoxyl- and hydroxyl groups.

The spectra of benzoquinones have 2 or 3 distinct maxima depending upon their substitution. The second maximum is shifted to longer wave-lengths mainly by substitution /Flaig, Ploetz and Küllmer 1955, Flaig and Salfeld 1958/. Substitution in the order methyl, methoxyl, hydroxyl, phenyl, secondary amino group shifts the absorption of p-benzoquinone derivatives to increasingly longer wave-lengths. Nearly no shift occurs in the case of toluquinone after substitution by a second methyl group in m- or p-position. Normally the size of aliphatic substituents has no marked effect on the shift of the second maximum.

The effect of the other groups as second substituents in m- or p-position is larger than in the case of methyl groups. However, substitution by the mentioned groups in o-position effects a larger shift than in m- or p-position. When o-xyloquinone is substituted by additional methyl or methoxyl groups as a 3. or 4. substituent, little effect on the shift of the second maximum is observed. o-Benzoquinones absorb at longer wave-lengths than the p-benzoquinones. Substitution in 3-position has a greater effect on the shift than in 4-position.

The 2. maximum of substituted dimer quinones is in the same wave-length range as the corresponding monomer, which is substituted by an aliphatic group on the carbon atom through which the rings are linked /Flaig and Salfeld 1958/.

It is unlikely that all phenolic units in the molecule of humic acids are present in the form of quinones, because these are not very stable against further oxidation. If quinonoid groups are present to a larger extent, they would presumably effect a broad maximum in the absorption curve in a range between 300 to 500 nm. This is not observed; therefore other effects must exist.

5.12 Radicals.

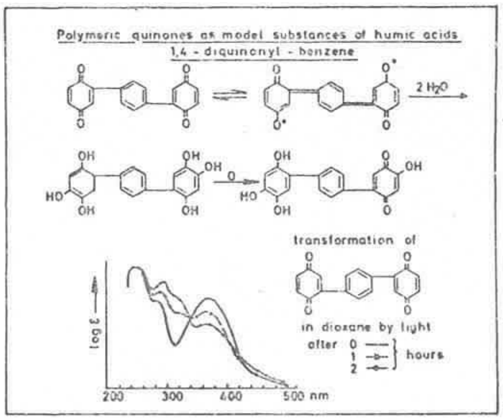


Fig. 10.: Polymeric quinones as model substances of humic acids, 1,4-diquinonyl-benzene.

We made some model experiments to explain the absence of distinct maxima in the absorption curve of humic acids. According to the UV-spectrum, 1,4-diquinonyl-benzene seems to be present in an o- and p-quinonoid configuration in solution /Ploetz 1955/. The o-configuration may be defined as a biradical and is very reactive. A corresponding hydroxyhydroquinone which can be partially reoxidised to a hydroxyquinone is formed by addition of water. A deeply coloured, intramolecular quinhydrone is formed. Diquinonyl-benzene is disproportionated in organic solvents by light. The formed mixture of substances show similar, nearly monotone increasing spectra as they are known from humic acids isolated from soils /Frömer 1938a,b, 1941 and later on many others/.

Steelink and Tollin /1962/, Steelink /1964/ demonstrated by electron spin resonance, that humic acids contain radicals. This result agrees well with the assumption that the humic acids are intramolecular quinhydrones. Measurements
ıd Mücks /1966/, Kleist /1967/ established that the radicals (semi-
e stabilized by mesomery and are responsible for the colour of humic
ıby, the humic acids would have the properties of an electron exchang-
Brigge, Lawson, Scruton and Wards /1965/ explain absorptions in IR-
rown coal humic acids by hydrogen bridge linkages between the hydrogen or phenolic hydroxyl groups and the carbonyl groups of quinones. Hitherto no quinone group could be found by reducing acetylation /Farmer and Morrison 1960/. Otherwise Martin, Dubach, Mehta and Deuel /1963/ assume that the carbonyl group of fulvic acids from podsol, which cannot be reduced by sodium boron hydride ($NaBH_4$), may belong to quinone groups.

5.13 Heterocyclic ring system.

A bathochromic effect may be caused by the oxygen, nitrogen or sulphur atoms in ring systems, which may occur in humic acids. The extent of the bathochromic effect of these elements is shown in the case of 4 dyestuffs with comparable chemical constitution.

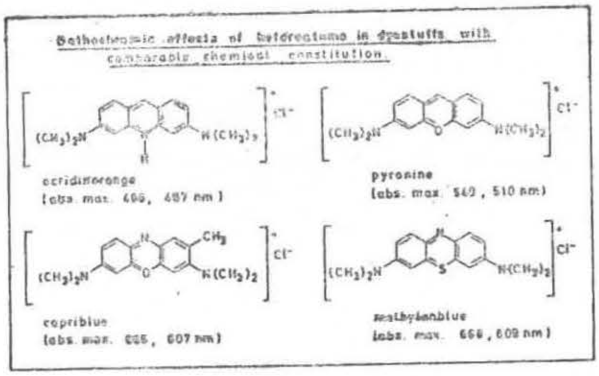


Fig. 11.: Bathochromic effects of heteroatoms in dyestuffs with comparable chemical constitution.

The influence of the shift of the absorption maxima increases in the order nitrogen, oxygen, sulphur and corresponding combinations. The range of absorption of these compounds is at longer wave lengths than that of the mentioned benzoquinone compounds.

5.2 Electronic spectra of humic acids.

In the following figure the absorption spectra of different humic acids and of fulvic acids are depicted schematically. The curves of absorption are not linear, as could be shown by more exact measurements (comp. Salfeld 1965).

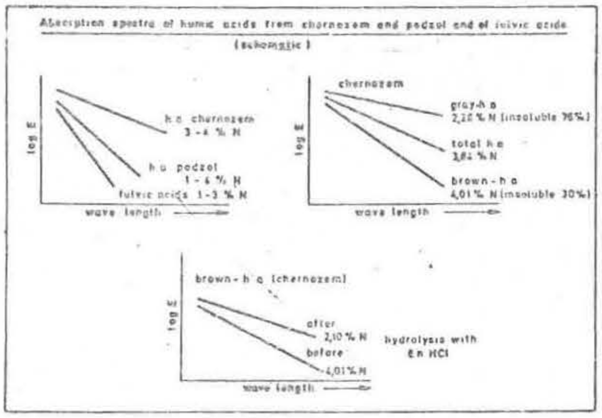


Fig. 12.: Absorption spectra of humic acids from charnezem and podsol and fulvic acid (schematic).

Tab. 3. Distribution of nitrogen in different humic acids and their b- and g-fractions.

	Podzol (Thyxengrube)		Chernozem (Sprendlingen) (accord. to Scharpenseel and Krausse 1962)	
Total-N (el.dial.)	0.85 %		3.13 %	
not a.a.-N	0.71 %		1.98 %	
	b.h.a.	g.h.a.	b.h.a.	g.h.a.
% fraction	71.9	28.1	85.5	14.5
Total-N in %	0.92	0.70	3.27	2.25
a.a.-content in %	1.04	0.65	8.78	2.55

Chernozem (Schöppenstedt) (accord. to Flaig, Scheffer and Klamroth 1955, Bremner 1955

Total-N (el.dial.)	3.84	
	b.h.a.	g.h.a.
Total-N in %	4.01	2.29
Nitrogen distribution after hydrolysis ref. to total-N		
Insoluble N in %	30.9	76.4
Soluble in %	69.1(100)	23.6(100)
NH_3-N in %	9.8(14.2)	7.7(32.6)
α-NH_2-N in %	37.5(54.3)	10.5(44.5)
Residue of hydrolysis in % of the fraction	58.3	79.6
Total-N in %	2.10	2.05

b.h.a.=brown humic acids, g.h.a.=gray humic acids, a.a.=amino acids

As a rule, humic acids from chernozems rich in nitrogen have not only higher absorptions, but also stronger absorptions at longer wave-lengths than the humic acids from podsols poor in nitrogen. The stronger absorption of humic acids from chernozems may be caused by the higher content of non-hydrolysable nitrogen, which may have a higher content of bathochromic heterocyclic compounds, than that in podsol humic acids. The total nitrogen content cannot play an important role in light absorption, since it is found largely in proteins, which do not absorb in the visible range.

The fraction of brown humic acids of chernozems has a higher nitrogen content than the fraction of gray humic acids. However, the absorption of the fraction of gray humic acids is stronger in the total range, than that of the fraction of brown humic acids.

It may be, therefore, that the residue of hydrolysis of the fraction of gray humic acids contains more heterocyclic, bathochromic components.

The absorption of the residue of hydrolysis of the fraction of brown humic acids is stronger in the total range than that of the fraction of the original brown humic acids, although the nitrogen content of this fraction (4,01 % N) is higher than that of the residue of hydrolysis (2,01 % N).

According to Kleist and Mücke (1966) the higher extinction of the fraction of gray humic acids is due to a higher content of radicals.

It seems that the absorption of fulvic acids is due more to oxidised phenols than to heterocyclic components with properties of dyestuffs, since these bathochromic components would show stronger absorption at longer wave-lengths. The absorption of fulvic acids is less in the range of longer wavelengths. The nitrogen content of fulvic acids is generally lower /Kononowa 1966/ and the carbonyl content higher /Schnitzer 1965/ than that of humic acids.

There is a voluminous literature on light absorption of humic substances. Recently Kumada /1965/ and very recently Orlov and Grindel /1967/ published summaries of work dealing with this problem.

The current theory concerning the colour of humic substances has been developed according to the results of chemical research, but it must be supplemented by further research and the isolation of corresponding compounds.

6. Particle form, size and weight of humic acids.

6.1 Random polymerization and effect of nitrogen.

The polymerization of phenolic units in the presence of nitrogenous compounds leads to the formation of the dark coloured higher molecular weight, humic acid like substances. The proposed enzymatic random polymerization of tyrosine to melanin under oxidising conditions can be used for the explanation of the processes which occur during the formation of humic acids. Melanin too is a higher molecular substance, and the polymerization leading to its formation occurs through quinonoid intermediates as is the case in the formation of humic acids.

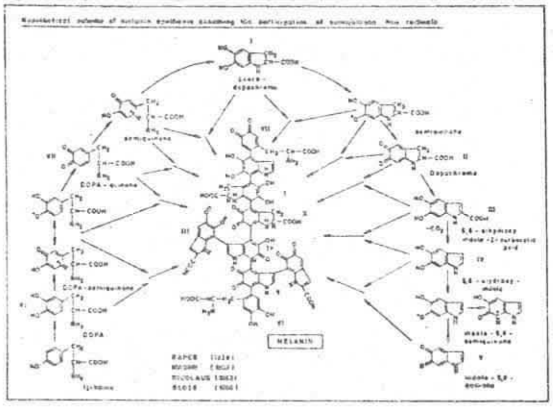
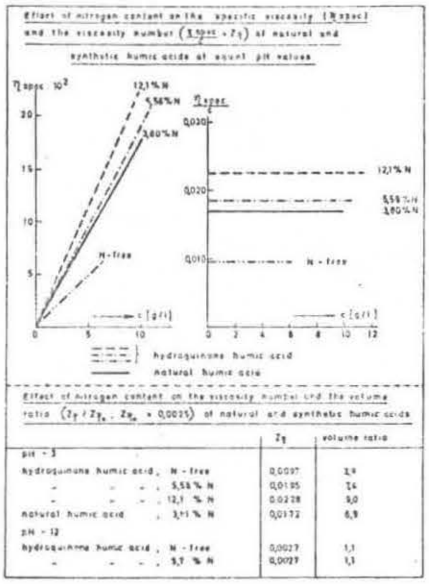


Fig. 13.: Hypothetical scheme of melanin synthesis assuming the participation of semiquinone free radicals.

The random polymerisation of tyrosine to melanin is a relatively simple process compared with the formation of humic acids. The formation of melanin starts from tyrosine as a single substance and indole-5,6-quinone, the end product of oxidation of tyrosine is known. This scheme shows, that not only the quinone formed on oxidation, but also that all intermediates participate in the formation of the three-dimensional, high molecular weight melanin /Blois 1965/.

Similar but much more complicated processes occur during the formation of humic acids from phenolic units and nitrogen compounds. The processes of polymerisation are more complex since several phenols and several nitrogenous components such as proteins, peptides, amino acids, amines and perhaps also ammonia participate in the formation of the polymers.

By random polymerization mostly spherically shaped colloids are formed. We made measurements of the viscosity of model humic acids from hydroquinone, which had been oxidized in alkaline solution in the presence of variable amounts of ammonia. The synthetic humic acids therefore contained different amounts of nitrogen. We concluded from the requirement of volume - that is the measured viscosity number to the theoretical viscosity number of 0,0025 according to Einstein - , that the humic acids are not compact spherically shaped colloids. They have pores and internal spaces and possess a structure which is comparable with that of a sponge /Flaig and Beutelspacher 1954/.



Effect of nitrogen content on the viscosity number and the volume ratio (Z_η / Z_{η_e}, Z_{η_e} = 0,0025) of natural and synthetic humic acids

	Z_η	volume ratio
pH = 3		
hydroquinone humic acid, N - free	0,0097	3,9
- - - , 5,58 % N	0,0195	7,6
- - - , 12,1 % N	0,0228	9,0
natural humic acid , 3,41 % N	0,0172	6,9
pH = 12		
hydroquinone humic acid , N - free	0,0027	1,1
- - - , 5,7 % N	0,0027	1,1

Fig. 14.: Viscosity number as a function of the nitrogen content of hydroquinone humic acids.

In the case of hydroquinone humic acids, the viscosity number increases with an increase in nitrogen content at pH = 3. The higher nitrogen content effects a stronger linkage inside the structure of the particles. For both nitrogen-containing and nitrogen-free humic acids the requirement of volume is nearly 1 at pH = 12. We suppose that the hydration is larger in acid then in alkaline solution.

If one transfers these results to natural humic acids it is not necessary for the relation between viscosity number and total nitrogen content to exist, since analytically determined α-amino nitrogen contributes less to the crosslinking than the nitrogen which is non-hydrolyzable and partly bound in heterocyclic form. This is the reason for the exact relationship between nitrogen content and viscosity number in the case of hydroquinone humic acids; deviations will occur in the case of natural humic acids.

6.2 Investigations with the electron microscope.

It is often possible to determine the particle form and size of colloids with the electron microscope.

Fig. 15.: Humic acids isolated from chernozem at pH 3,5.

The aggregation to the ramified structure and the simultaneous formation of coacervates occurs at pH-values of 3.5, whilst the humic acids are dispersed in alkaline suspension to nearly single particles.

Fig. 16.: Humic acids isolated from chernozem at pH 8.

We showed some years ago /Beutelspacher 1952, Flaig and Beutelspacher 1951/ that the particle diameters of humic acids from chernozem reprecipitated 23 times are in order of 50 to 100 Å. The numerous reprecipitations reduced the ash content to below 1 % and allowed only high molecular weight materials to remain in the suspension.

According to the mentioned requirement of volume and the deduced spongy structure of the humic acids, it can be concluded from Wo.Ostwald /1928/ that the units of humic acids are also hydrated inside the colloids. The strength of hydration varies with environmental conditions. From our measurements with the ultracentrifuge we found an increase in density of the particles in 0,2 N sodium chloride solution at a pH-value of 5,0 compared with a suspension in water. We assume that this is due to a decrease of hydration, which leads to a change in the partial specific volume.

Hydration shells are also dimished mechanically by ultrasonic vibration. We could show by ultrasonic treatment of suspensions of humic acids that the electron optical density increased and the coacervates were partly degraded.

Fig. 17.: Humic acids (pH=3,5) and ultrasonic treatment.

6.3 Investigations with the ultracentrifuge.

In published accounts of work in which the ultracentrifuge has been used, only values for the sedimentation constant have been reported /Piret, White, Walther and Madden jr. 1960, Scheffer, Ziechmann and Schlüter 1958/. The sedimentation constant alone without the diffusion constant does not allow a statement about the particle weight of high molecular weight substances to be made. The above-mentioned authors stated that an exact determination of the diffusion constant is impossible, since the preparation of monomolecular humic acids has not, or has only seldom, succeeded. For our investigations we used a new ultracentrifuge, which was built and improved by Beutelspacher in cooperation with the firm M. Christ, Osterrode. The special advantages of this centrifuge are a more intensive source of light for investigations of coloured substances, a higher constancy of rotation, a higher constancy of temperature (± 0,01° C) and the application of a new optical system for analytical purposes.

Apparative errors, such as the contra diffusion to the centre of rotation, which are described in measurements of humic acids /Scheffer, Ziechmann and Schlüter 1958/, and which are evidently based on the inconstancy of the temperature, no longer occur.

6.31 The influence of hydrogen ion concentration on the determination of the sedimentation and diffusion constant as well as on particle weight, diameter and friction of humic acids in water suspensions and in the presence of 0,2 M NaCl.

The humic acids were isolated from a "marsch" soil with 0,1 N sodium hydroxide solution. The soil was acidified before extraction and washed with water. After acidifying the extraction solution the humic acids were reprecipitated 4 times and electrodialyzed using 0,10 milliampere and 110 volt. The fractions were taken from the center of the dialyzer at the mentioned pH-values and immediately investigated with the ultracentrifuge.

The time dependent course of sedimentation of the electrodialised samples of humic acids in water suspension was determined in the ultracentrifuge by measuring the movement of the refractive index gradient with a schlieren optic.

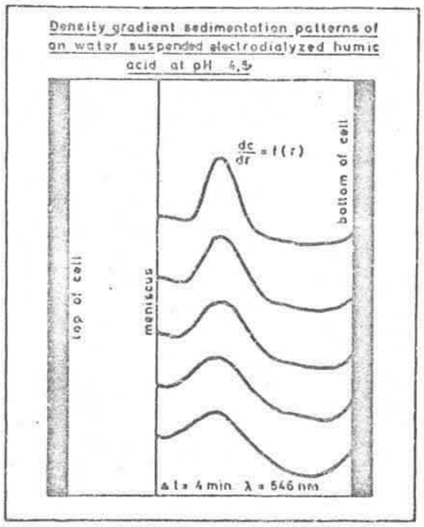


Fig. 18.: Density gradient sedimentation patterns of a water suspended, electrodialysed humic acid pH 4,5.

The sedimentation diagrams, recorded by photography at 4 minute intervals, show that humic acids consist of particles of different size. One part appears as a high molecular weight substance in form of a Gaussian distribution pattern, whilst the other accumulates at the bottom of the moving boundary cell under the influence of gravitation at 50.000 rpm in between some few minutes as a macromolecular substance and causes a strong increase of the refractive index. The Gaussian distribution curve shows an asymmetric course by the presence of different sizes of molecules. This is shown especially by the descending part of the curve.

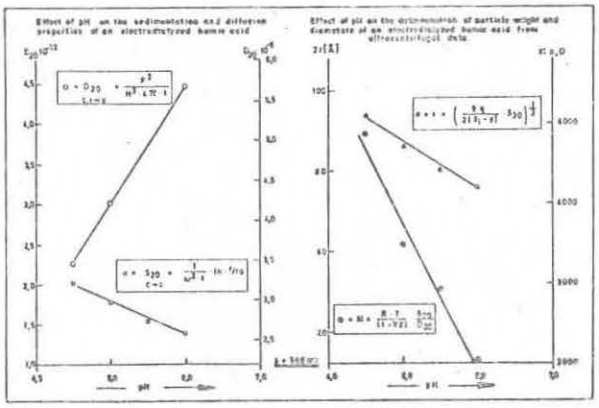


Fig. 19.: a) Effect of pH on the sedimentation and diffusion properties of an electrodialyzed humic acid.

b) Effect of pH on the determination of particle weight and diameter of an electrodialyzed humic acid from ultracentrifugal data.

The next graph shows that the sedimentation constant increases and the diffusion constant decreases with increasing hydrogen ion concentration (fig. 19.).

The particle weights calculated according to the formula of Svedberg /1934/ increase from 2 030 at pH = 6.0 to 4 850 at pH = 4.5.

The diameters of the particles also increase from 78 Å to 92 Å with increasing hydrogen ion concentration.

Tab.4.: Effect of pH on sedimentation (s_{20}), diffusion (D_{20}), particle weight ($M_{s,D}$), particle diameter (2r) and the friction coefficient (f/f_o) of humic acid in water and in 0,2 M NaCl suspension.

pH	$S_{20} \cdot 10^{-13}$	$D_{20} \cdot 10^{-6}$	$M_{(s,D)}$	2r(Å)	f/f_o
6,0	1,38	5,66	2030	76	1,0
5,5	1,53	4,32	2950	80	1,1
5,0	1,77	4,21	3490	86	1,1
4,5	2,01	3,45	4850	94	1,1
5,0 in 0,2 M NaCl	4,46	0,48	77000	138	1,5 x)
4,5 in 0,2 M NaCl	4,71	0,65	60400	142	1,2 x)

x) Corrected for the amount of salt added /Elias 1961, p. 126/

In these investigations it is possible to estimate the particle form by means of the friction coefficient. Therefore we calculated the friction coefficient from the pH-dependent values of the sedimentation and diffusion constants. It can be seen from the ratio f/f_o (f_o = molar friction coefficient of globular particles according to the Stokes formula /1849/, f = measured coefficient), the values are in the range between 1,0 and 1,1. It can be concluded, therefore, that the humic acids are spherical colloids. This result confirms our former investigations with the electron microscope.

The effect of charge must be recognized when the dissociation of high molecular weight substances is studied, since the polyions and counter ions possess different charges and different sedimentation and diffusion coefficients due to large differences in their particle sizes. Therefore, a charge gradient occurs during sedimentation leading to a decrease in the rate of sedimentation of humic acid polyions. An addition of neutral salts decreases the primary charge effect, and the necessary electron neutrality for an unhindered sedimentation is reached. The minimum amount of neutral salt which must be added depends upon the concentration of the polyelectrolytes. The empirical values for a 1 % solution of protein are in the range of 0.2 molar sodium chloride. We used this concentration of sodium chloride for our measurements.

Humic acids may be compared with globular proteins to a certain extent. Like globular proteins, humic acids contain acidic groups and nitrogen which is assumed to have a basic function. Furthermore, it may be estimated from the determined values of α-amino-nitrogen and from the amino acids bound in peptide - like fashion that humic acids have a protein content of about 5 %.

The results of experiments with 2 electrodialyzed samples are depicted in tab.4. The values have been measured at pH = 4.5 and 5.0 after addition of sodium chloride. It should be noted that remarkable differences occur in salt containing suspensions in comparison with water suspensions. The sedimentation constant increases from 1.77 to 4.46×10^{-13} at pH = 5.0 and from 2.01 to 4.71×10^{-13} at pH = 4.5.

The values of the diffusion constant are especially influenced by the addition of sodium chloride. A comparison of the diffusion constants determined in water shows that the values decrease from 4.21 to 0.48×10^{-6} at pH = 5.0 and from 3.54 to 0.56×10^{-6} at pH = 4.5. The sedimentation constant is more than doubled on addition of sodium chloride, while the diffusion constant decreases about ten-fold.

Due to the considerable decrease in the diffusion constants, particle weights of 77,000 versus 3,490 at pH = 5.0 and 60,400 versus 4,850 at pH = 4.5 were determined for the main part of the suspension. The high values are not essentially different from the particle weights estimated in the electron microscope studies. The diameters of the particles deviate little from those which were determined in pure water suspension. The values obtained were 138 and 142 Å respectively.

The friction ratio $f:f_0$ moves to 1.2 and 1.5 and is therefore a little higher than that in water suspension. Therefore, it can be concluded that the morphology of humic acids shows little deviation from the ideal sphere.

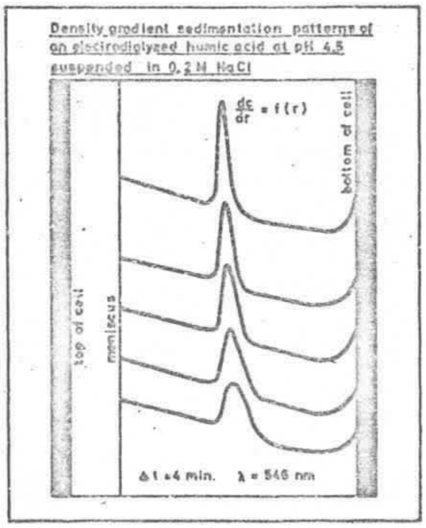


Fig. 20.: Density gradient sedimentation pattern of an electrodialysed humic acid at pH 4.5 suspended in 0.2 M NaCl.

If one compares the sedimentation diagrams of humic acids with and without addition of salt, which have been obtained under the same conditions, it can be established that the polydispersity in the salt-containing suspension is not so as marked as in a water suspension. The half-width value of the Gaussian distribution curve is remarkably smaller, and the precipitation at the bottom of the cell is reduced. But the right part of the Gaussian distribution curve always deviates more from the frequency maximum in the course of the progressing sedimentation. This may be evidence in support of our opinion that particles with a higher weight are present.

Several authors believe that humic acids could be characterised simply by determining the sedimentation constant by ultracentrifugation, and that it is possible to estimate particle weights by consideration of a variable sedimentation coefficient at a constant value of the diffusion constant.

According to our results, the above procedures would lead to misinterpretations, because the diffusion constant shows a strong variation due to environmental conditions. A two-fold increase in the sedimentation constant and a ten-fold decrease in the diffusion constant, relative to the primary values, cause a 15 to 20 fold increase in particle weight. These deviations are not negligible in particle weight determinations. We can conclude, therefore, that particle weights can be used for characterising humic acids only when both constants are measured.

7. Summary.

I have discussed some problems of chemical composition and physical properties of humic substances with regard to their phenolic and nitrogenous structure units.

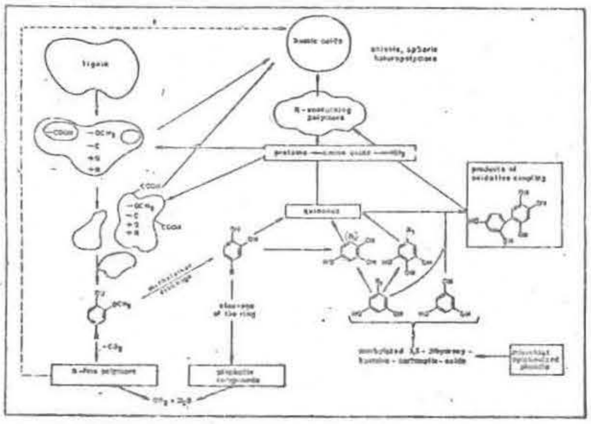


Fig. 21.: Contribution of phenolic and nitrogenous structure units to the chemical composition and physical properties of humic substances.

The scheme depicted above is concerned only with the problems discussed and therefore gives no information about other processes which also occur during humification.

The analytical data show that there are essentially two sources of phenols for the formation of humic substances in nature.

(1) Phenols of mainly resorcinol and phloroglucinol type which are synthesized by microorganisms, contribute to the composition of humic acids. These substances are altered by transformation to phenols which can be oxidized to quinones and by reactions with nitrogenous compounds or oxidative coupling with quinones.

(2) The formation of phenols from lignin occurs mainly by microbial degradation,

in which the cleavage of the aromatic ring plays an important role and the structure of the lignin molecule is disrupted. Large or small degradation products are formed, which contribute to the composition of humic substances by reactions with nitrogenous compounds in all phases of the degradation.

In the course of the above-mentioned reactions, the formation of heterocyclic compounds which may contribute to the colour, an essential property of humic substances, also occurs.

The chemistry of the mentioned structural units leads to an elucidation of the processes by which higher molecular weight substances are formed. The humic acids are anionic, spherical heteropolymers and seem also to have properties of electron exchangers. After apparative improvements of an ultracentrifuge, contributions to the knowledge of particle form, size and weight of humic acids were made. The determination of sedimentation and diffusion constants is always necessary for characterizing humic acids according to the mentioned physical properties.

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